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XPRESS DNA™ Tissue Mini Kit

CAT No. : 1404-TI-50
1404-TI-25

User Manual

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Kit Contents:

The XpressDNA™ Tissue Mini Kit is available to process 25 and 50 samples.

XpressDNA™ Tissue Mini Kit		
Components	XPD1404-TI-50	XPD1404-TI-25
	Quantity	
XpressDNA™ Lysis Buffer	40 ml	20 ml
XpressDNA™ MagNa Mix	18 ml	10 ml
XpressDNA™ Wash Buffer	10 ml	5 ml
Proteinase K (Lyophilized)	20 mg/ml	10 mg/0.5 ml
Proteinase K Buffer	1 ml	0.5 ml
RNase A Solution	20 mg/ml	10 mg/0.5 ml

Shipping and Storage:

All components of the XpressDNA™ Tissue Mini Kit are shipped at room temperature. The RNase A Solution has to be stored at 4 °C. The lyophilized Proteinase K can be stored at room temperature for 6 months. Before use reconstitute the Proteinase K with the buffer provided and store the reconstituted Proteinase K at 4 °C upto 2 months. For long term use, make aliquots of reconstituted Proteinase K and store at -20 ^{0}C.

Introduction

The XpressDNA™ Tissue Mini Kit allows rapid and efficient purification of genomic DNA from both fresh and frozen tissues (animal or human). The genomic DNA is extracted using the XpressDNA magnetic nanoparticles-based technology. This approach facilitates easy, inexpensive DNA isolations and avoid time consuming steps like centrifugation and column separation. The extracted DNA is suitable for use in downstream applications including PCR, restriction enzyme digestion, etc.

The XpressDNA Technology:

The XpressDNA magnetic nanoparticles-based technology works with a swappable charged solid surface which facilitates nucleic acid purification. Under suitable buffering conditions, the magnetic nanoparticles acquire a positive charge enabling the negatively charged DNA molecules to bind to them leaving the contaminants in the solution.

System Specifications:

Starting Material: 50–100 mg tissue

Elution Volume: 100 µl

DNA Yield: 10-20 µg

Before You Begin

It is important to read this section before starting the protocol to avoid non significant results.

General Information

For the maximum recovery of the DNA, go through the following information:

- Use consumables and equipments that are DNase-free
- Maintain a sterile environment to avoid DNase contamination
- Perform recommended wash steps to avoid contaminants in the extracted DNA

Sample Processing

To obtain good results use fresh tissue or tissue that has been immediately frozen and stored at –20 °C or –70 °C. Repeated freeze-thaws of stored samples should be avoided, since this leads to shearing of the DNA. Use of poor quality starting material can also result in reduced yield and quality of the purified DNA.

The XpressDNA™ Tissue Mini Kit has been standardized using tissues like spleen, liver, kidney, muscle and fish fin. The kit is also optimized for the extraction of genomic DNA from ethanol preserved fish tissues.

Handling of MagNa Mix

- Store the MagNa mix at room temperature
- Before use mix the magnetic nanoparticles on vortex mixer
- When added to the sample, mix either by inverting the tube or by pipetting. Try to avoid bubbles while mixing
- Never dry the magnetic nanoparticles for too long after ethanol wash as it will reduce the efficiency of magnetic nanoparticles
- Carefully remove the supernatant without disturbing or removing the magnetic nanoparticles
- Do not freeze the magnetic nanoparticles as it will be damaged and cannot be used for DNA purification
- Do not reuse the magnetic nanoparticles

MagnaStand

The MagnaStand consists of a base station and a tube holder molded together. The tube holder can accomodate 12 microcentrifuge tubes. The base of the MagnaStand contains six rare-earth magnets, that comes in contact with the microcentrifuge tube when in-use.

Elution

For eluting the DNA, use nuclease-free water (not supplied). The volume of nuclease-free water can be changed according to the required final concentration. Be sure to use the nuclease-free water volume not less than the volume required to resuspend the magnetic nanoparticles.

Safety Information:

Follow the safety guidelines below when using the XpressDNA Tissue Mini kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear suitable lab coat, disposable gloves, and protective eye wears
- If any spill occurs, clean with a suitable laboratory detergent and water
- If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

Protocol: Extraction of Genomic DNA using XpressDNA™ Tissue Mini Kit

This protocol is designed for the isolation of genomic DNA from fresh, frozen and ethanol preserved tissues.

Materials Required:

User Supplied

- Tissue sample
- Nuclease-free water
- Mortar & pestle
- XpressDNA MagnaStand or other magnetic separation rack
- Sterile 1.5 ml microcentrifuge tubes

- Vortex mixer
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)
- Water bath or heat block

Components Supplied with the Kit:

- XpressDNA Lysis Buffer (TI1)
- XpressDNA MagNa Mix (TI2)
- XpressDNA Wash Buffer (TI3)
- Proteinase K (Lyophilized)
- Proteinase K Buffer
- RNase A Solution

Starting Material:

Use this procedure to isolate genomic DNA from 50-100 mg tissue. Do not use more than 100 mg of tissue. To obtain high yields of DNA and minimize DNA degradation, use fresh samples or freeze the sample immediately in liquid nitrogen after collection.

Things to do before starting the protocol:

- ❒ Set the water bath or heat block at 56 ^{0}C.
- ❒ Wash Buffer (TI3) is provided as concentrate. Before the first use, add 96-100% ethanol as indicated on the bottle.
- ❒ Reconstitute the Proteinase K in Proteinase K Buffer (volume mentioned on Proteinase K vial) and store at 4 ^{0}C after each use.

Procedure:

1. Obtain the required tissue freshly from source or remove the tissue from storage. If using frozen tissue, equilibrate it to room temperature. Cut and weigh 50-100 mg of tissue sample (depends on the type of homogenization). After use, store the remaining tissue according to the standard methods.

   For ethanol preserved tissue, take 50-100 mg of tissue and put it in a DNase-free 1.5 ml microcentrifuge tube containing 500 µl of nuclease free water. Incubate for 1 hour at room temperature to rehydrate the sample. Then remove the water using a pipette and proceed to Step 2 for tissue

disruption and homogenization.

2. Tissue disruption and homogenization can be performed by the following methods:

2a. Using a mortar and pestle in liquid nitrogen:

Place 50-100 mg tissue in a mortar containing liquid nitrogen and grind the tissue using a pestle. Transfer the tissue powder into a DNase-free 1.5 ml microcentrifuge tube containing 750 µl Lysis Buffer. Proceed to step 3.

2b. Using a mortar and pestle without liquid nitrogen:

Place 50 mg tissue in a mortar containing 250 µl Lysis buffer and grind the tissue using a pestle. Transfer the tissue lysate into a DNase-free 1.5 ml microcentrifuge tube containing 500 µl Lysis Buffer. Proceed to step 3.

2c. Using a sterile blade:

Place 50 mg tissue in a sterile petri dish and cut the tissue into small pieces using a sterile surgical blade to increase the efficiency of tissue lysis. Transfer the minced tissue into a DNase-free 1.5 ml microcentrifuge tube containing 750 µl Lysis Buffer. Proceed to step 3.

3. Add 20 µl of RNase A to the lysate and mix by inverting the tube. Incubate the tube at room temperature for 10 minutes.

4. Add 20 µl of Proteinase K to the tube and mix by inverting the tube.

Note: The incubation time after the addition of Proteinase K varies with the type of tissue homogenization.

5. Incubate the sample according to steps 5a, 5b, 5c.

5a. **Homogenization using motor and pestle with liquid nitrogen:** Incubate the sample for lysis at 56 ^{0}C for 30 minutes or until the lysate appears clear.

5b. **Homogenization using motor and pestle without liquid nitrogen:** Incubate the sample for lysis at 56 ^{0}C for 45 minutes or until the lysate appears clear.

5c. **Homogenization using sterile blade**: Incubate the sample for lysis at 56 ^{0}C until the lysate appears clear (approximately 1-2 hours).

Note: The lysis time varies with the type, age and amount of tissue being used. For soft tissues, in 1-2 hours the lysis is completed, and for fibrous tissue or rat tail, an overnight incubation at 56 ^{0}C is optimal.

6. Add 350 µl of XpressDNA MagNa Mix to the digested tissue sample and

mix by inverting the tube 5 times. Incubate at room temperature for 5 minutes.

Note: If using pipette, use 1 ml pipette and set it to 900 µl to mix the sample. Make sure that the entire volume is pipetted to avoid foaming as this may result in shearing of the DNA as well as sample loss.

7. Place the tube in the XpressDNA MagnaStand for 2 minutes or until the solution appears clear.
8. With the tube on the XpressDNA MagnaStand, carefully remove and discard the supernatant. Take care not to disturb the separated magnetic nanoparticles.
9. Add 500 µl of XpressDNA Wash Buffer to the tube and invert the XpressDNA MagnaStand up and down gently five times to wash the magnetic nanoparticles.
10. With the tube on the XpressDNA MagnaStand, carefully remove the supernatant and discard. Take care not to disturb the separated magnetic nanoparticles.
11. Repeat steps 9 - 10 for a total of two washes.
12. After completely drying the separated magnetic nanoparticles, remove the tube from XpressDNA MagnaStand and add 100 µl of nuclease-free water to the tube and gently pipette up and down 10 times to resuspend the magnetic nanoparticles.
13. Incubate the tube at 65 ^{0}C for 5 minutes.
14. Place the tube in the XpressDNA MagnaStand for 2 minutes or until the solution appears clear.
15. With the tube on the XpressDNA MagnaStand, carefully remove the supernatant containing the DNA to a sterile 1.5 ml microcentrifuge tube. Take care not to disturb the separated magnetic nanoparticles.
16. Discard the used magnetic nanoparticles.
17. Store the purified DNA at –20 ^{0}C or use immediately for the desired downstream application.
18. Avoid repeated freezing and thawing DNA. Store the purified DNA at 4 ^{0}C for short term use or aliquot the DNA and store at –20 ^{0}C for long term storage.

Troubleshooting Guide

Problem	Cause	Solution
Low DNA yield or No DNA recovered	Incomplete lysis	Start with lesser amount of tissue. Do not use more than 100 mg of tissue. Add appropriate amount of Proteinase K for lysis The tissue should be completely immersed in the lysis buffer. Increase the incubation time at 56 ^{0}C.
	Poor quality of tissue sample	Properly store the tissue sample. Process fresh tissues or frozen tissues that has been immediatly frozen after collection.
	Improper handling of XpressDNA MagNa Mix	Vortex the MagNa Mix thoroughly before use. Carefully pipette the entire volume to avoid foaming as this might lead to sample loss. Take care not to disturb the magnetic nanoparticles when on the MagnaStand.
	Inefficient elution	The volume of nuclease-free water used should be enough to resuspend the magnetic nanoparticles. Incubate at 65 ^{0}C for 5 minutes.

Problem	Cause	Solution
DNA is sheared	Repeated freeze-thaw of tissue samples	Avoid repeated freezing and thawing of tissue samples.
	Poor quality of tissue sample	Do not use old tissue samples. This often yields only degraded DNA.
	Contamination of work place and consumables with DNases	Maintain a sterile environment while working. Use DNase-free consumables.
Inefficient performance of DNA in down stream applications	Ethanol carryover	Dry the separated magnetic nanoparticles (containing the DNA) to remove the ethanol after the wash steps.
	Salt carryover	Ensure that the correct amount of wash buffer is added and a total of two wash steps are performed.

APPENDIX

Accessory Products

Additional Products:

The table below lists additional products in pipeline available from MagGenome Technologies Pvt. Ltd. under the brand name XpressDNA for the isolation of genomic DNA from different sample types. For more information about these and other XpressDNA Kits, visit: www.maggenome.com or contact Technical Support.

Product	Quantity	Catalogue No.
XpressDNA Blood Mini Kit	50 Reactions	1401-BL-50
	25 Reactions	1401-BL-25
XpressDNA Bacteria Mini Kit	50 Reactions	1402-BA-50
	25 Reactions	1402-BA-25
XpressDNA Fecal Mini Kit	50 Reactions	1406-FE-50
	25 Reactions	1406-FE-25
Magna Stand, 12 positions	1	XPD-SD-12